Effect of Shear Rate Variation on Apparent Viscosity of Human Blood in Tubes of 29 to 94 \( \mu \text{m} \) Diameter

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In order to test the hypothesis that the increase of vascular resistance observed \textit{in vivo} at low flow rates is due in part to blood rheological properties, the apparent viscosity of human blood was measured in small tubes in a range of shear rates. Pressure–flow relationships were obtained in vertical glass tubes (29 to 94 \( \mu \text{m} \) i.d.) perfused with blood at hematocrits between 0.13 and 0.65. Viscosity of blood and plasma was calculated using Poiseuille’s law. With the exception of data obtained in the largest tube at a hematocrit of 0.6, relative blood viscosity was found to be independent of shear rate in the range between 1 and 120 s\(^{-1}\). Microscopic observation revealed pronounced red cell aggregation at low shear rates. Velocity profiles obtained by the use of fluorescence-labelled red cells showed increased blunting with decreasing shear rate. The Fahraeus–Lindqvist effect was evident in a reduction of viscosity with tube size at a given feed hematocrit. The observed constancy of apparent blood viscosity with decreasing shear is attributed to the opposing effects of a cell-depleted marginal layer and red cell aggregation or deformation in the cell core. The findings indicate that the increase of vascular resistance at low arterial pressure cannot be explained by shear-dependent changes of apparent blood viscosity observed in macroviscometers. (\textit{Circulation Research} 1986;59:124–132)

It is well known that blood possesses significant nonlinear rheological properties. Studies in large scale viscometers\(^1\)-\(^3\) have shown a disproportionate increase in apparent blood viscosity with decrease of shear rate, especially in the range below 50 s\(^{-1}\). Relatively few studies have been undertaken under flow conditions that might be more similar to those existing in the vascular system. Published data on tube flow appear to confirm the general trend of an increase in the apparent viscosity of blood with decreasing shear rate.\(^4\)-\(^7\) Little data on the effects of shear rate on apparent viscosity are available for tubes in the diameter range of the arterioles and venules that represent the primary sites of pre- and post-capillary resistance in the vascular system. Predictions of \textit{in vivo} viscosity changes with alteration of perfusion conditions are therefore largely based on data obtained from viscometric studies in large viscometers; such data, however, may not adequately reflect the rheological behavior of blood in the peripheral circulation.

The present study was undertaken to investigate the changes of apparent blood viscosity with shear in a range of tube diameters and shear rates that are relevant to the microcirculation. Such data are useful as a reference for \textit{in vivo} studies, since direct determinations of blood viscosity in microvessels are complicated by additional factors such as leukocyte–endothelium interactions.\(^8\)

Materials and Methods

Human whole blood was perfused through glass capillaries with inner diameters between 29 and 94 \( \mu \text{m} \) and lengths between 35 and 65 mm. All diameters were determined by end-on microscopy. The capillary was part of a perfusion system (Figure 1) mounted vertically on the stage of a horizontal microscope (Leitz Ortholux). The upper (i.e., upstream) end of the glass capillary was glued into the open tip of a 2-ml plastic syringe barrel, which served as a feed reservoir; the orifice of the capillary was located approximately 3 mm above the bottom of this reservoir. The downstream end of the capillary was glued into a larger bore glass cross piece connecting the capillary with a horizontal glass tube, which was used for determination of flow rate (flow measuring tube). The flow measuring tube was in turn connected to a length of flexible tubing attached to a second reservoir. The entire system was filled with saline degassed to remove microscopic air bubbles. The diameter of the flow measuring tube was at least 6 times that of the capillary. Therefore, the contribution of this tube to total resistance of the system was considered negligible compared to that of the glass capillary. The flow measuring tube contained a small volume of \( n \)-butanol (stained with Sudan-black), which served as an indicator of fluid movement. The pressure drop across the system was set by adjusting the position of the second saline-filled reservoir relative to the upstream reservoir.

The perfusion system was mounted on the microscope stage such that the image of the vertical capillary could be observed and recorded using a low-light-level video camera (Siemens KB5) and a video cassette re-
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FIGURE 1. Schematic drawing of the experimental set-up. Details in text.

corder (Sony U-matic, VO-5800PS). A second video camera (Grundig FA 123) was used to simultaneously record, on the same video tape, the movement of the n-butanol meniscus in the flow measuring tube.

The perfusion system was cleaned with cleaning solution (10% Edisonite) added to the upstream reservoir, while a water pump vacuum line was attached to the downstream end of the flow measuring tube. Forced perfusion with cleaning solution was performed for several runs or overnight as necessary to obtain system performance within the limits described below. All solutions used were prefiltered through 1.2-μm filter membranes.

Each experiment started with determination of the pressure–flow relations for saline solution. These were obtained by varying the driving pressure and determining the movement of the n-butanol meniscus with the use of a video x-y-position analyser (For-A, VPA-1000). Zero pressure was verified optically by observing the movement in the capillary of a few red cells added to the saline solution. Volume flow rates through the capillary tube were calculated from the meniscus movement and the diameter of the flow measuring tube. Experiments were not started or continued unless pressure–flow curves for saline were linear with a zero pressure–flow intercept and agreed with predictions from Poiseuille's law within approximately 5%.

Human blood was obtained by venipuncture from 6 healthy staff members and anticoagulated with 5 mg/ml EDTA (ethylenediaminetetraacetate). The hematocrit of the blood was varied by removal or addition of appropriate volumes of plasma separated by centrifugation. In one study (31 μm tube, feed hematocrit 0.45) the buffy coat was removed after centrifugation. Pressure–flow curves were obtained first with saline and plasma followed by blood samples of different hematocrit. The blood in the reservoir was stirred gently but continuously in order to prevent sedimentation. In each experimental run the sequence of driving pressures used was varied randomly. In most experiments 6 pressure steps in the range 10–800 mm H2O were used to obtain blood pseudo shear rates (defined below) from about 1 s⁻¹ up to about 120 s⁻¹. In some experiments measurements were repeated at a given pressure and hematocrit. Perfusions with saline or plasma were performed between runs with different blood samples to verify constant capillary conductivity. The experimental arrangement allowed us to examine the capillary tube over most of its length and, in most instances, enabled us to view the exit orifice. Experimental data were not accepted if blood cells were seen adherent to the tube walls or exit orifice. Temperature was continuously measured with a probe on the microscope stage. Experiments were conducted at ambient temperatures between 24 and 30°C, and data were corrected to 26°C using the temperature–viscosity relationship for water. Within any experiment the temperature was maintained at 1.5°C. Temperature changes were less than 0.2°C within any measurement at a given pressure. Depending on the magnitude of volume flow, measurements of meniscus movement were taken over periods of 1.5–25 minutes.

Red cells were fluorescence labelled by incubation with a solution of FITC (E. Merck, Darmstadt, Art.Nr. 24546). After removal of plasma and buffy coat the red cells were washed twice with filtered saline and subsequently incubated for 60 minutes in a phosphate-buffered solution of FITC (1 mg/ml) at a pH of 7.8 and a solution osmolality of approximately 270 mosm/kg. Following incubation the cells were washed several
times in saline and then resuspended in their native plasma. Labelled cells were added to the blood samples used for perfusion experiments; the labelled cell fraction was between 0.2 and 1% of all red cells. Microscopic examination of wet mounts revealed normal red cell shape; labelled red cells were incorporated in nonlabelled red cell rouleaux.

**Data Analysis**

Pseudo shear rate in the capillary tube ($\dot{\gamma}$, mean velocity in tube diameters/s) was calculated from the rate of meniscus movement and the ratio of cross-sectional areas of the flow measuring tube and the capillary. The pseudo shear rate is given by the equation

$$\dot{\gamma} = \frac{Q}{R^2 D}$$

where $Q$ is the volume flow rate, $D$ is the diameter, and $R$ represents the radius of the perfused glass capillary. For a Newtonian fluid, $\dot{\gamma}$ would be equivalent to one eighth of the wall shear rate.

Computations of apparent viscosity ($\eta_{app}$) were based on Poiseuille's law:

$$\eta_{app} = \frac{\pi \Delta P R^4}{8QL}$$

where $\Delta P$ is the driving pressure and $L$ is the length of the capillary tube. Relative blood viscosity was obtained by dividing the apparent blood viscosity by the viscosity of the plasma.

Velocity profiles were determined by single-frame analysis of the video recordings taken during epi-illumination in the fluorescence microscope (Leitz Ploemopak 2.3). The objective was focussed on the median plane of the tube. The depth of focus was such that cells above and below the median plane were also included in the analysis. The distance travelled by each of the observed labelled cells between two successive video frames (time interval 20 msec) was determined using the x-y-position analyser (For-A, VPA-1000), and converted into a cell velocity. Velocities obtained were normalized with respect to the maximum velocity observed in the center of the tube.

Data were fitted by linear or power regressions, and correlation coefficients were obtained. The $t$ test was used for analysis of significance.

**Results**

A linear relationship between flow and pressure was apparent in the 31-\textmu m tube as the driving pressure was varied between 25 and 650 mm H$_2$O with a feed hematocrit of 0.29 and 0.64 (Figure 2, top left). The lines shown were derived by linear regression analysis. The slopes decreased with increasing hematocrits. The pressure–flow relationships in the 41-\textmu m tube were also linear for the same range of pressure and feed hematocrits of 0.15 to 0.66 (Figure 2, top right). Similar results were obtained in the 59-\textmu m tube with feed hematocrits of 0.20 to 0.65 (Figure 2, bottom left) and in the 94-\textmu m tube at hematocrits between 0.2 and 0.6 (Figure 2, bottom right). Not shown in Figure 2 are the data obtained in tubes of 29, 30, and 66 \textmu m diameter, which showed similar results. Regression lines for all hematocrits in all tubes except the 94-\textmu m tube showed intercepts that were not significantly different from zero ($p > 0.05$). Correlation coefficients were 0.99 or higher.

The absolute viscosity of plasma at 26°C was calculated from the pressure–flow curves and found to range from 1.49 to 1.71 cp for samples from 6 different subjects. No dependence of plasma viscosity on shear rate was observed. The relative viscosity of the blood samples compared to plasma is plotted in Figure 3 as a function of pseudo shear rate. Although in some cases the slope of the linear regression was significantly ($p < 0.05$) different from zero (31-\textmu m tube, hematocrit 0.37; 94-\textmu m tube, hematocrit 0.2, 0.6), a systematic variation of blood viscosity with pseudo shear rate was not apparent. In the 94-\textmu m tube, however, a strongly nonlinear increase of relative viscosity was observed at a feed hematocrit of 0.6. These data were therefore fitted with a power function. With this exception, the findings indicate a remarkably constant apparent blood viscosity over the measured range of pseudo shear rates and feed hematocrits.

In some trials not depicted in the figures we noted a systematic increase of apparent blood viscosity as the flow rates were reduced. Close inspection of the tube in these instances revealed aggregates of blood cells (mostly platelets), adhering to the tube, usually at the exit orifice. The latter formed an obstruction that partially covered the orifice, except at higher flow rates, when it was forced away. This artifact was not seen in the experiments depicted in Figures 2 and 3.

As noted in the methods section, platelets and leukocytes were removed from one blood sample with a hematocrit of 0.45 and used in the 31-\textmu m tube. There was no apparent effect of the presence of platelets or leukocytes on the measured blood viscosity except, as noted above, when platelet aggregates formed at the outflow orifice.

The relationship between apparent blood viscosity and feed hematocrit is shown in Figure 4 (left panel). Since, with one exception, apparent viscosity was independent of shear rate, the mean viscosity was taken for each tube at each hematocrit; for the 94-\textmu m tube and a feed hematocrit of 0.6, the viscosity obtained at the highest shear rate was taken. A curvilinear hematocrit–viscosity relationship is apparent for each of the tubes, represented in Figure 4 by a power regression. The steepness of the obtained relationships decreases with reduction in tube diameter. Also shown for comparison are the viscosity values obtained in a large-scale viscometer at a shear rate of 170 s$^{-1}$. The right panel of Figure 4 shows the relationship between relative apparent blood viscosity and tube diameter, which obtain from the regressions in the left panel at feed hematocrits of 0.40 and 0.45. Also shown are the measurements of Fahraeus and Lindqvist performed on three blood samples whose hematocrit was not giv-
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FIGURE 2. Pressure-flow relations obtained in four capillary tubes with plasma and blood samples with different feed hematocrits (data points with different symbols). Dashed lines indicate linear regressions for plasma, solid lines for blood samples. Regression coefficients decreased with increasing feed hematocrit, correlation coefficients were 0.99 and higher.

Discussion

The present studies show a remarkable constancy of apparent blood viscosity over a wide range of flow rates in tubes with internal diameters between 29 and 94 μm. In view of the consistent observations of increased viscosity at low shear rates reported both in glass tubes and in macro-viscometers by other investigators, these findings were unexpected. To reconcile these findings with earlier reports, a critical analysis of methodology seems appropriate.

In our study the most critical problem is accurate measurement of flow rate. Since the saline and n-butanol were immiscible, the meniscus remained intact except when the measuring tube became contaminated and this condition was readily apparent. Temperature changes, which were small, would not lead to a systematic error. Moreover, a nonlinear artifact in our measuring system would be required to just offset the hypothesized nonlinear behavior of the blood. However, such an artifact would be expected to cause a nonlinear pressure-flow relationship for saline and plasma, which was not seen. Thus, we are led to conclude that our measurements are a valid reflection of the flow properties of blood in our system.

A key feature of our system is that we used a vertical tube while horizontal tubes have been used in most previous studies. In a horizontal tube sedimentation of red cells or red cell aggregates into the lower portion of the tube could occur at low flow rates. While this might lead to an increase of local hematocrit and, thus, local viscosity in the lower portion of the tube, the opposite effect would occur in the upper portion. The overall effect of sedimentation would probably be an increase in the measured apparent viscosity in the tube due to the nonlinear relation between hematocrit and viscosity. We should also note that larger tubes were

en in the original publication; since these authors had anticoagulated native blood samples by adding 0.5 ml citrate solution to 10 ml blood, the final hematocrit was presumably in the range between 0.4 and 0.45. As seen from this figure, the data confirm the presence of the Fahraeus–Lindqvist effect in the present measurements.

Observing the microscopic flow field during the experiment and during video tape replay we observed red cell aggregates in the center of the capillary tubes at low, but not at high, shear rates. Further corroboration of this was seen in the velocity profiles. The profiles shown in Figure 5 were obtained in the 66-μm tube at a feed hematocrit of 0.5 and pseudo shear rates of 0.69, 3.35, 11, and 26.4 s⁻¹; the corresponding cell velocities in the tube centerline were 0.064, 0.31, 0.59, and 1.19 mm/s. As evident from Figure 5, the velocity profiles exhibited a significantly more blunted appearance at low compared to high shear flow.
used in all but one of the previous studies and the shear rate effect may also be dependent on tube size (see below). Adhesion of cells to the tube wall was noted in another study but was not considered to be significant. Based on our experience, this phenomenon could have contributed in some instances to the increased apparent viscosity noted by other workers at low flow.

In previous rheological studies performed in large-scale viscometers, blood viscosity was found to be shear independent only if both the aggregation tendency and the physiological deformability of the red cells had been experimentally abolished. In the present experiments, however, red cell aggregation was quite obvious from the video-microscopic observations at low perfusion pressures and the blunted velocity profiles, and there is no reason to believe that the red cells were rendered undeformable.

Since red cell aggregation, which is considered to be the major cause of increased blood viscosity at low shear, did take place in our experiments, the observed shear independence of apparent viscosity may be explained, in large part, by two opposing consequences of aggregation. On one hand, the formation of rouleaux and larger cell networks at lower shear rates leads to an increased blunting of the velocity profile in the tube center, which is indicative of decreasing local shear rates and increased local viscosity. On the other hand, red cell aggregation also leads to an increased width of the marginal cell-depleted layer of fluid in which most of the shear occurs. While the first phenomenon increases local viscosity in the center of the tube, the enlarged cell-depleted layer at the periphery reduces local viscosity in that region. The complex interplay between red cell aggregation and the associated formation of a marginal plasma layer has been discussed by previous authors. These considerations led Fahraeus to predict a decreased viscosity at low shear. In addition, it might be expected that the lesser extent of red cell deformation at low shear also tends to increase apparent viscosity. It appears, therefore, that these phenomena compensate each other, such that the resultant viscosity remains constant over the entire range of shear rates. We consider this exact compensation to be fortuitous and note that in two instances (31-μm tube, Hct 0.37 and 94-μm tube, Hct 0.6) viscosity was dependent on shear rate. Our results may reflect the specific aggregation tendency and red cell deformability present in normal human blood. The variation of blood viscosity with changing shear rates may therefore be quite different in other species or if the aggregating tendency of human red blood cells is abnormal.

In contrast to the situation in the tubes used here, the viscosity increasing effect of aggregate formation dominates the overall behavior of the blood in a large viscometer at low shear, since the marginal cell-depleted layer is small compared to the gap width of the viscometer. Moreover, the walls of some viscometers are roughened to limit the formation of a cell-free
layer, thus facilitating the measurement of bulk rheological properties.\textsuperscript{15} Therefore, the difference between our findings and macroviscometric data appear explainable on a rheological basis.

The fraction of the tube cross section occupied by the cell-depleted marginal layer at the periphery of the tube decreases with increasing tube diameter. It may therefore be expected that the effectiveness of the marginal layer in compensating the increased viscosity resulting from cell aggregation at low shear will be less pronounced in tubes with large diameter. The present findings are therefore not inconsistent with the results of earlier studies of tube flow, in which an increase of viscosity at low shear rates was found in a larger tube diameter range.\textsuperscript{4,5,16} Since no data on the shear rate effect on apparent blood viscosity have been reported in a range of tube diameters between 30 and 10 \(\mu\)m, it can at present not be decided whether significant changes of viscosity with variation of shear rate will be present. However, as the tube diameter approaches that of the single red blood cells, the flow dependence of cellular deformation might be expected to induce a nonlinear behavior of blood viscosity.\textsuperscript{17,18}

The Fahraeus–Lindqvist effect, which has previously been observed in blood-perfused tubes by others,\textsuperscript{4,7} was quite evident in our studies, as is obvious from the data in Figure 4 (left panel), which show lower viscosities in the tubes used here compared to the results obtained in a viscometer.\textsuperscript{3} Although the hematocrit of the blood samples used by Fahraeus and Lindqvist\textsuperscript{9} was not precisely known, good agreement between their viscosity values and the ones found here is obvious from Figure 4 (right panel). In view of the diameter dependence of blood viscous behavior it appears that the Fahraeus–Lindqvist effect is sensitive to shear rate both in rather large (diameter > 100 \(\mu\)m) and in small (diameter < 10 \(\mu\)m) tubes, but not in the intermediate diameter range studied here, except at rather high hematocrit levels.

Since red cell packing in the tube center resulting from aggregation of cells at low shear rates may lead to a finite maximum cell concentration in the central core, the width of the marginal layer must also decrease with increasing hematocrit.\textsuperscript{19} It is therefore also conceivable that the compensatory effect of the marginal layer on apparent blood viscosity at low shear rates is reduced at elevated hematocrits. The above explanation of the present findings is thus quite consistent with the observation of a significant increase of apparent blood viscosity in the largest tube and at the highest hematocrit used.

In vivo studies, particularly whole organ experiments, have generally shown an increase in calculated blood viscosity at low perfusion pressures or flow rates. The reported magnitude of these changes, however, differed considerably. Whittaker and Winton
found that the apparent viscosity of blood in the dog hind limb rose about 10% as perfusion pressure was reduced from 88 to 44 mm Hg. A similar change was observed by Gustafsson et al in a comparable pressure range. Djojosugito et al found that blood viscosity doubled with pressure reduction to 10 mm Hg. Baekström et al found that blood viscosity in the same preparation rose 200–300% when flow was reduced to less than 1 ml/min/100 g.

Interpretation of blood viscosity changes derived from whole organ perfusion experiments is difficult, because the flow conditions vary between the various vascular compartments. For instance, the effect of hematocrit alterations on flow resistance has been shown to differ between the arterial and venous and the microvascular compartments as a result of the Fahraeus–Lindqvist effect. In addition, high shear conditions normally exist in the precapillary vessel section, while the venous vessels exhibit low shear flow even under normal perfusion conditions. On the other hand, the contribution of postcapillary vessels to total vascular resistance is low. Therefore, even substantial changes of blood viscosity in the low shear venous vessels may not be adequately detected by measurements of total vascular resistance. It may thus be appropriate to consider the different vascular compartments separately, if the effective changes of blood viscosity with flow rate are to be evaluated.

Studies of venous resistance in skeletal muscle during reduced flow indicate an inverse relationship to flow: Resistance rose two to three fold as arterial pressure was reduced from normal levels to 20–30 mm Hg. Also, in exercise venous resistance decreased 50–75% in skeletal muscle as flow increased two to three fold. Since these effects were attenuated by hemodilution, shear rate dependent changes in blood viscosity were invoked to explain the changes in venous resistance. Microcirculatory studies by Lipowsky et al indicate a strong shear rate dependence of blood viscosity in single arterioles and venules at low shear rates. Also, studies by House and Johnson indicate that the pressure gradient in the venular network of skeletal muscle is not strictly dependent on flow rate. This finding was interpreted to indicate that blood viscous resistance varied inversely with flow rate.

The current studies indicate that apparent blood vis-
cosity does not change in glass tubes of size comparable to the venules and a large portion of the arteriolar network over a range of flow rates of physiological interest. The studies appear to rule out formation of red cell aggregates as a causal mechanism for the observed increases of venous resistance and of apparent blood viscosity seen in microcirculatory and whole organ studies with reduced flow rates. This conclusion is certainly valid at normal hematocrit, since in vivo studies consistently show that microvessel hematocrit is lower than systemic.31-34 If systemic (and thus microvessel) hematocrit is elevated, nonlinear rheological properties may become relevant, particularly in the larger microvessels. This follows from the present findings in the 94-μm tube. Inasmuch as these findings were obtained with normal human blood, extrapolation to other species or to situations with altered red cell aggregation tendency must be made with caution.

A variety of other rheological effects unique to the in vivo situation have been invoked to be responsible for this effect, such as plugging of capillaries and other terminal microvessels by red cell aggregates or platelets23 and by white cells.35,36 It is also well known that white cells tend to marginate and to travel near the vessel wall as flow rate is reduced.37-39 If such a phenomenon occurs in the venular network, a condition would obtain where white cells could preferentially interact with and adhere to the endothelial cells of the venules and increase resistance as shear rate is reduced.40-42 These potential explanations require further study.

For an application of the results reported here to blood flow in microvessels in vivo it should also be noted that flow conditions in a complicated network may not be readily comparable to those in a single unbranched tube with a length-to-diameter ratio in the order of 103. In the vascular system, frequent vessel branching or junctions may prevent the development of a fully established marginal plasma layer. Thus, in vivo apparent blood viscosity may not be adequately determined in an in vitro system such as used here. We suggest that a close approximation of in vivo flow geometry is required for a definitive in vitro assessment of blood rheology relevant to the microcirculation.

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References


**Key Words** • apparent blood viscosity • shear rate • tube flow • red cell aggregation • pressure–flow relation • Fahraeus–Lindqvist effect • velocity profile
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